100 YEARS AGO

The San Francisco correspondent of the *Daily Mail* reports that the people of Santa Barbara, a county of southern California, are terror-stricken owing to the increasing frequency and severity of the earthquake shocks, of which there were seventy-five from July 27–31. The most destructive was that at the town of Los Alamos, at 1.20 a.m. on July 31. All the brick buildings were thrown to the ground, but the frame buildings generally escaped serious injury except to their windows... The shock lasted thirty seconds, and seems to have had a spiral motion. Goods were hurled from the shelves of the stores and piled in the middle of the rooms; even heavy desks were tossed about. The inhabitants ran into the streets in a panic, for in the morning between 7.25 and 7.30 there were additional shocks, and just before nine two more. … The earth continues to tremble at intervals, and the countryside is said to be changing appearance.
From *Nature* 7 August 1902.

50 YEARS AGO

Dr. Conant points out that we are now dealing with the consequences of a new social phenomenon. Until the advent of nuclear weapons, developments in defensive or offensive weapons depended on the special application of the publicly known facts and principles of physics and chemistry. Any new scientific knowledge involved had no revolutionary consequences for the advance of science. The flowering of the whole field of nuclear physics, on the contrary, depended on the expenditure of a large sum of public money, justified in the first instance in terms of the destructive power of a weapon required in a desperate global struggle. Inevitably nuclear physics, thus born of a war-time necessity, was marked from its birth with the secrecy which was anathema to nineteenth-century men of science. The advance of one whole area of science was thus equated with national security; and Dr. Conant insists that the assumption that science knows no frontier is automatically cancelled and we must face the consequences. We must distinguish sharply between the special position of nuclear science and that of the other fields of physics and chemistry. This is the first step towards preventing the spread of undue secrecy and thereby assisting the progress of science and promoting the welfare of the international community of science.
From *Nature* 9 August 1952.

the temporal sequence of equidistantly spaced laser pulses is transformed into a sequence of unit-charge current pulses. For a laser-pulse repetition rate of 82 MHz, the expected current is 13.1 pA, which agrees well with the value observed by Zrenner *et al.* of 12.6 pA. The experiment is effectively a single-electron turnstile device — one electron passes per operation cycle in a quasi-deterministic fashion.

But several problems must be overcome if this is to be the basis of viable quantum-computing technology. To give just two examples, Zrenner *et al.* found that, as the excitation power is increased, the oscillations become considerably damped, seeming to die away. The origin of this damping and a strategy for overcoming it need to be worked out. Second, it must be shown that coherent processing occurs with subsequent laser pulses of the exciton quantum state generated by the first laser pulse.

Nevertheless, looking to the future of coherent optoelectronics, the work of Zrenner *et al.* will no doubt prove significant. Their single-electron turnstile may become one of the building-blocks of a new generation of optoelectronic devices of unprecedented performance. ■

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Structural biology

Calcium callisthenics

N. Michael Green and David H. MacLennan

The Ca^{2+}-ATPase is one of the best characterized of the pumps that move ions across cell membranes. The latest snapshot of the pump in another of its many conformations is a major step in understanding its dynamics.

The action of skeletal muscle depends on the flow of calcium ions into and out of a storage compartment — the sarcoplasmic reticulum — in muscle cells. Release of Ca^{2+} into the cytosol stimulates muscle contraction. The reverse flow to replenish the Ca^{2+} store and permit muscle relaxation is controlled by a membrane protein, the Ca^{2+}-ATPase, which occurs in other cells but in muscle cells constitutes up to 90% of the membrane protein. In their paper on page 605 of this issue[1], Toyoshima and Nomura provide a spectacular view of the conformational changes that take place between this pump in its calcium-free and calcium-bound states.

Ca^{2+}-ATPase is part of a large family of enzymes, all of which contain a specific amino-acid residue, aspartic acid, that is phosphorylated by ATP only in the presence of the appropriate cation. The cation concerned enters the pump from the cytoplasmic side, is trapped within the membrane and is released on the luminal or extracellular side through gates that are controlled by conformational changes in the enzyme. The kinetic cycle for the Ca^{2+}-ATPase is shown in Fig. 1. It consists of a complex, reversible sequence of phosphorylation and dephosphorylation.

Two years ago, Toyoshima and colleagues published[2] a crystal structure of the muscle Ca^{2+}-ATPase, SERCA1a. This both confirmed widely held concepts[3] about the mechanism of a Ca^{2+} pump, and provided fresh insights into its action. This structure showed the Ca^{2+}-bound form, $E1Ca_2$ (Fig. 2b), with its three cytoplasmic domains well separated, but joined to one another or to membrane helices by extended links (this contrasts with the compact structure of these domains that was observed in an earlier, lower-resolution structure for E2P; ref. 4). Only the catalytic P domain was anchored more firmly, by substantial extensions of the transmembrane α-helices M4 and M5. Calcium was bound in the transmembrane domain to two high-affinity sites formed by the precise juxtaposition of M4, M5, M6 and M8, with the 12–14 ligands to the Ca^{2+} ions linking these helices together.

To complete the cycle and release Ca^{2+} to the lumen of the sarcoplasmic reticulum, ATP is bound in the jaw formed by the N and P domains, which closes, allowing phosphorylation of the crucial aspartic acid (D351). A slow rearrangement of phosphoenzyme

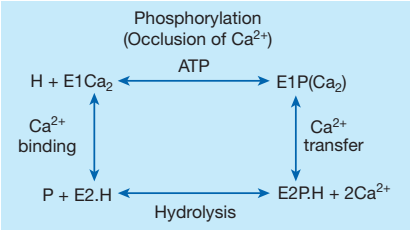

Figure 1 **The basic reaction cycle of Ca^{2+}-ATPase. Binding of Ca^{2+} and phosphorylation alternate with transfer of Ca^{2+} and hydrolysis. The structural transformation discussed in the text is that between E2H and $E1Ca_2$.**

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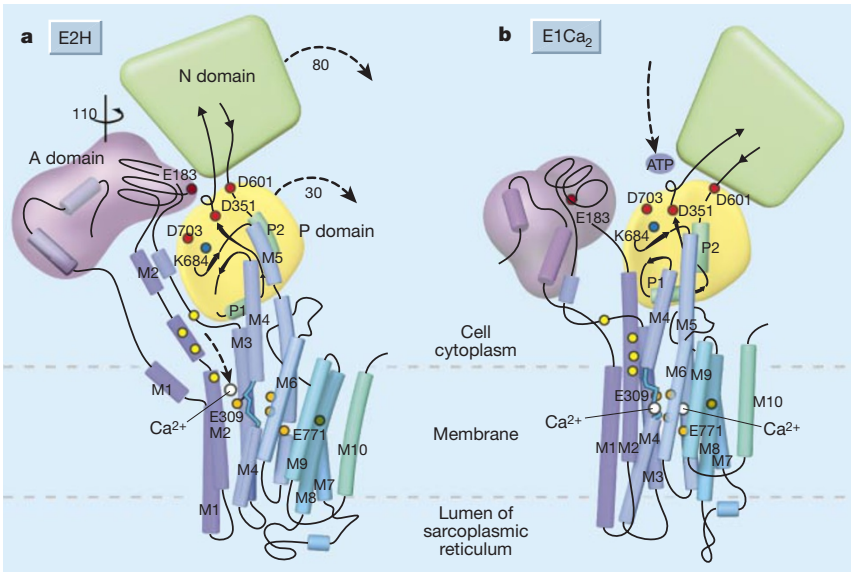

Figure 2 **Structure and function in a Ca^{2+} pump. Shown here are the Ca^{2+}-free (E2H) and Ca^{2+}-bound ($E1Ca_2$) states of SERCA1a, illustrating the effects of Ca^{2+} binding on the pump structure. Three domains, P (phosphorylation), N (nucleotide-binding) and A (actuator), lie in the cytosol. The P domain, with N inserted, connects the tops of transmembrane helices M4 and M5. Two β-strands, which continue from these helices, form additional anchors that are part of the central β-sheet. This domain includes a cluster of critical catalytic residues: the phosphorylation site D (aspartic acid) 351, D703 and D601, and K (lysine) 684. Some of the Ca^{2+} ligands (orange) on transmembrane helices M4, M5, M6 and M8, and some of the polar or negatively charged residues (yellow) that line a possible Ca^{2+} entry pathway, are shown in relation to bound or unbound Ca^{2+} (white).** a, **The E2H state, as determined in the new work[1]. Entry to the Ca^{2+} sites is blocked by E (glutamic acid) 309 in M4. The P, N and A domains form a compact cluster. The A domain is rotated to meet the P domain and carries E183 into the catalytic site, where it may be involved in hydrolysis of E2P.** b, **The $E1Ca_2$ state. In the crystal structure[2], the cytoplasmic domains have moved apart (they are known to be mobile in solution). ATP can bind in the cleft between N and A, but because it induces a new conformation its location is only partly defined.** (a, **Based on refs 1, 2;** b, **based on ref. 2.**)

$E1P(Ca_2)$ disrupts the Ca^{2+}-binding sites, opening the luminal gate. This allows water to enter the catalytic site and hydrolyse the phosphoenzyme E2P, generating the E2H conformation. A channel then opens to allow cytoplasmic Ca^{2+} access to the binding sites to re-form $E1Ca_2$.

In the new paper[1], Toyoshima and Nomura describe how they have captured SERCA1a at 3.1 Å resolution in the E2 configuration (Fig. 2a). The pump is protonated[5], and there is good evidence that protons are counter-transported[6], like potassium ions in another well-characterized ion pump, the Na^+/K^+ pump. The protonated residues are not defined, but four carboxyl groups that provide Ca^{2+} ligands are likely candidates. In comparison with $E1Ca_2$, there are complex changes in the transmembrane Ca^{2+}-binding site and the 'stalk' connections, which are amplified even further by movement of the cytoplasmic domains, which gather to form a compact cluster (Fig. 2a).

In the dephosphorylated segment of the cycle ($E2H \leftrightarrow E1 \leftrightarrow E1Ca \leftrightarrow E1'Ca \leftrightarrow E1'Ca_2$), the pump loses protons, binds one Ca^{2+}, undergoes a conformational change, and then cooperatively binds a second Ca^{2+}. The structural correlates of this transition are illustrated in Fig. 2. Large-scale movements of the N, P and A domains occur with little internal change. They are coupled to changes in tilt and position of helices M1–M6, but M7–M10 are almost unmoved. The bottom section of M5, below glutamic acid (Glu) 771, remains fixed, packing against M7. But the middle section, with its cytoplasmic extension bonded to the L67 loop, tilts back, straightening the helix and carrying the top section — an integral part of the P domain — through a 30° rotation. The largest movements of Ca^{2+} ligands are in M6, which becomes more helical on binding Ca^{2+}, several ligands rotating through 90°. The M4 helix moves down by 5 Å, carried by the tilting of M5. The net result is that the Ca^{2+} ligands, which are dispersed in E2H, come together to form compact sites in $E1Ca_2$.

These changes stabilize a new configuration of the surrounding helices, and the effects are transmitted to the P domain, to M1, M2, M3, and to the A domain. Parts of the structure are likely to be thermally labile, the molecule being stabilized in E2H by hydrogen bonds between the protein domains and by nonpolar interactions (see Fig. 1, right, on page 606), and in $E1Ca_2$ by multiple ligands to Ca^{2+}. In the cytoplasm, binding of Ca^{2+} leads to an open domain structure, which can rearrange further after phosphorylation. The coordinating role of the P domain is clear from its many interactions with N, A and the transmembrane and stalk domains.

The E2H structure highlights the access routes for Ca^{2+}. An early model[7] of the transformation between E1 and E2 proposed that Ca^{2+} was bound to exposed luminal sites in E2 or to cytoplasmic sites in E1. Lack of competition between these sites suggested[8] that access to the lumen was confined to stage E2P. Consistently, in E2H, closure of luminal loops L34 and L78 blocks access from this side. On the cytoplasmic side, Glu 309 in M4 rotates to lie at the bottom of a negatively charged, aqueous pocket in E2H (ref. 9), blocking access to the main Ca^{2+}-binding sites. A small movement of Glu 309, possibly triggered by ionization, might open this entrance and allow Ca^{2+} to enter the binding sites. This would be consistent with a gating role, proposed to explain the properties of mutants of Glu 309 in the Ca^{2+} pump and Glu 327 in the Na^+/K^+ pump[10].

Further progress will, ideally, require determination of the structures for the ATP-bound ($E1ATPCa_2$) and phosphorylated ($E1P(Ca_2)$ and E2PH) states. However, these intermediate conformations are labile, and stabilizing ligands, such as thapsigargin in the E2H structure, will be needed, making them structural guides rather than true intermediates. Although it may be possible to define further conformational steps between binding of calcium and phosphorylation, it may be more realistic to represent the process as a balance between global conformations, tipped one way or the other by ligands or by phosphorylation. Toyoshima and Nomura[1] have illuminated one small step in the cycle of the Ca^{2+} pump. But their depiction of the dynamic interrelationships among the key residues and interlinked domains is a giant step forward in understanding the mechanism of the ATPase family that includes SERCA1a. ■

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